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Clinical and Biochemical Heterogeneity in Females of a Large Pedigree With Ornithine Transcarbamylase Deficiency Due to the R141Q Mutation

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A large family with ornithine transcarbamylase deficiency due to mutation R141Q was ascertained through a propositus who presented with acute neonatal hyperammonemic coma. Of 13 females at risk, 11 were evaluated clinically and had laboratory studies performed. Seven were found to be heterozygous for the mutation. Of these seven, five had chronic clinical symptoms and two were asymptomatic. None of the heterozygotes had elevated plasma ammonia on random testing. Of the five symptomatic females, three had markedly elevated plasma glutamine levels on random testing, while two had levels in the upper range of normal. Plasma citrulline and arginine levels were somewhat lower in the symptomatic individuals but still within the normal range. Five heterozygotes who were tested had either spontaneous orotic aciduria or elevated orotic acid following ingestion of allopurinol, whereas one unaffected female and one unaffected male had normal allopurinol tests. A higher than expected proportion of female heterozygous for the R141Q mutation were clinically and biochemically symptomatic but remained undiagnosed for many years. Plasma glutamine determination and allopurinol testing should be performed in females who present with a combination of relatively non-specific symptoms detailed in this report.

KEY WORDS: hyperammonemia, ornithine transcarbamylase gene, ornithine transcarbamylase deficiency, urea cycle, X-linked heterozygosity, clinical heterogeneity

INTRODUCTION

Males with complete deficiency of ornithine transcarbamylase (OTC) enzyme activity, the most common inherited defect in the urea cycle, usually present with severe neonatal hyperammonemia. They require intensive measures such as hemodialysis to prevent neonatal death and/or brain damage [Brusilow and Horwich, 1995]. In contrast, females heterozygous for severe mutations in the OTC gene can be asymptomatic, mildly affected, or severely affected, presumably due to variable mosaic patterns of OTC deficiency in their livers [Riccuiti et al., 1976]. In heterozygotes with partial deficiency, dietary, environmental, hormonal, and yet other poorly understood factors are likely to play important roles in disease expression and outcome. The available studies on females heterozygous for OTC deficiency [Batshaw et al., 1986; Hawks Arn et al., 1990] report data from many patients but are limited by genetic heterogeneity due to "private" mutations [Tuchman et al., 1996] and different genetic backgrounds. Thus, a significant portion of the variability observed in some of these studies could be related to the type of mutation affecting the subjects. We had the opportunity to study members of an unusually large pedigree affected by the R141Q mutation with respect to clinical presentation and biochemical parameters. We examined the correlation between the biochemical findings and clinical signs and symptoms in patients of this family.

PATIENTS

All patients studied are part of an extended family in which the abnormal OTC allele was transmitted for at

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least three generations. The pedigree is illustrated in Figure 1. The family was ascertained through the diagnosis of a male propositus who developed acute neonatal hyperammonemia on the second day of life. His plasma ammonia level reached 1,300 μM (normal <50), plasma glutamine, alanine and asparagine were very elevated and citrulline was undetectable. His urinary orotic acid was also very elevated at 142 mmol/mol of creatinine (normal <3.1). The OTC gene was analyzed by polymerase chain reaction of genomic DNA followed by single-strand conformational polymorphism (SSCP) and direct sequencing as previously described [Tuchman et al., 1992]. A mutation in exon 5, codon 141 (CGA>>CAA, arginine>>glutamine, R141Q), known to cause complete OTC deficiency [Lee and Nussbaum, 1989] was found. He was treated with hemodialysis, peritoneal dialysis, sodium benzoate, sodium phenylacetate, arginine, and citrulline. He recovered from the neonatal hyperammonemia and underwent liver transplantation at the age of 7 months.

The propositus had three maternal uncles and one maternal great uncle who died in the newborn period with no diagnosis. Pedigree analysis indicated that 13 females were at risk for OTC deficiency in this family. This number includes a maternal grandmother, mother, sister, four maternal aunts, and six female cousins. Shortly after the propositus was diagnosed, one of his maternal aunts contacted us regarding OTC deficiency in her six daughters. One of these female cousins had overt mental retardation, while several others had serious behavioral and learning disabilities. None carried a diagnosis despite repeated medical evaluations beginning in the newborn period. We were also contacted by the propositus' remaining three maternal aunts and the maternal grandmother. Two of the maternal aunts had healthy sons and one had no children by choice but was a strict vegetarian and suffered from depression. The propositus' mother was generally healthy but recounted an unusual episode of lethargy and confusion following the delivery of her son.

METHODS

The females at risk for OTC deficiency were interviewed and examined, and their previous records were reviewed when available. Plasma ammonia and amino acids were determined by routine methods. Urinary orotic acid analysis was performed by stable isotope dilution [McCann et al., 1995] prior to (once) and after (four times within 24 hours) ingestion of 300 mg of allopurinol as described. Genomic DNA was analyzed for the presence of a mutation in the OTC gene as described [Tuchman et al., 1992]. After the deleterious mutation was identified, carrier status for the R141Q mutation was confirmed or excluded using SSCP and/or sequencing of PCR products of exon 5 of the OTC gene as described [Tsai et al., 1993].

RESULTS

DNA testing confirmed that the maternal grandmother, mother, two maternal aunts, and three maternal

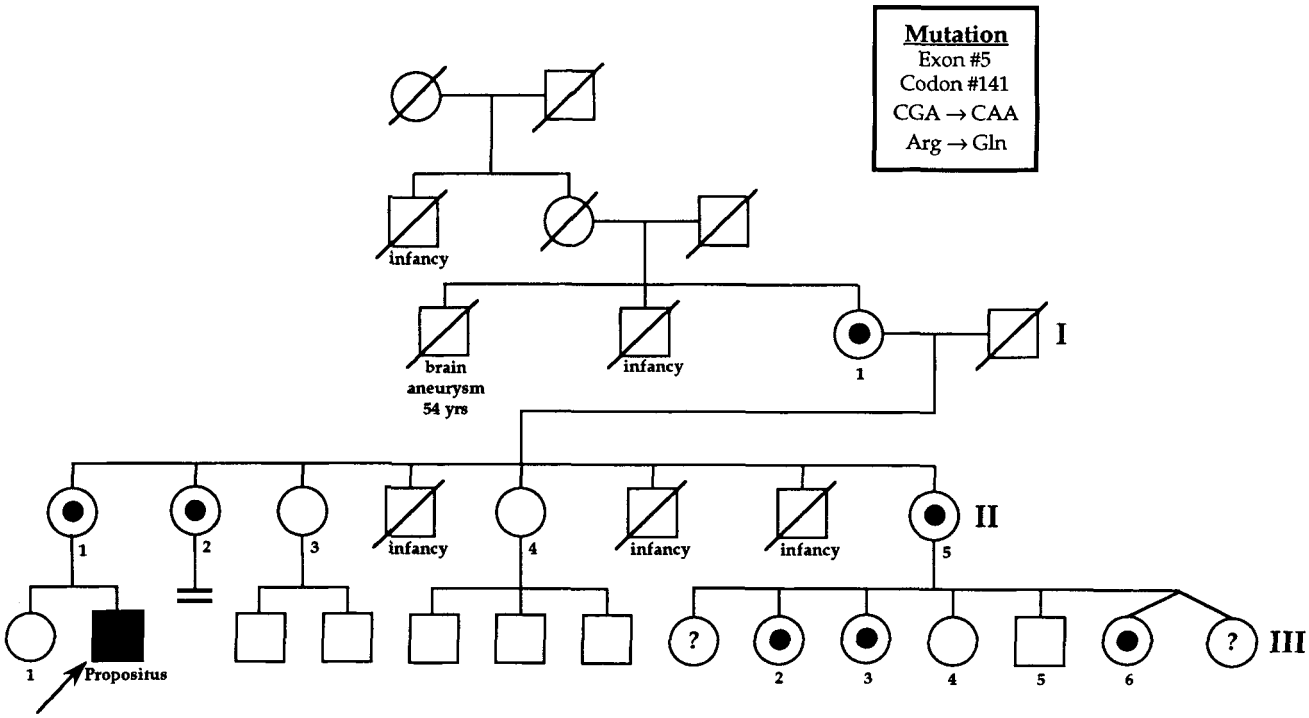

Fig. 1. Pedigree illustration of the family with OTC deficiency. Question marks indicate that no tests were performed to establish carrier status. All other relatives' genotypes have been established.

female cousins were heterozygous for the deleterious mutation (data not shown). The two remaining maternal aunts, the propositus' sister, one female cousin and one male cousin were found to carry normal OTC alleles. Two maternal cousins at risk refused to be tested.

Clinical Manifestations

Relevant clinical and biochemical observations are summarized in Table I. Five of the seven confirmed heterozygotes had clinical symptoms that could be attributed to hyperammonemia and/or arginine deficiency. Three (III-2, III-3, and III-6 in Fig. 1) age 15, 13, and 21 years, respectively, were more severely symptomatic; however, prior to testing for OTC carrier status, none had a specific diagnosis. Symptoms in female III-6 were particularly severe and included seizures, mental retardation, behavioral disorder, central hypotonia, cyclic vomiting, and abnormal hair texture. The clinical findings in female III-2 included learning disabilities, short stature, lethargy at birth, and abnormal hair texture. Behavioral problems and hair abnormalities were the main clinical findings in female III-3 and all three were avoiding protein rich foods. Two additional heterozygotes (II-5 and II-2) had depression and one (II-2) was a vegetarian and had abnormal hair.

Laboratory Findings

All heterozygous females had normal plasma ammonia levels on a single random sample. However, on concurrent plasma amino acid analysis, the three symptomatic female cousins (III-2, III-3, and III-6) described above had markedly elevated plasma glutamine levels above 1,000 μm/L, and either spontaneous orotic aciduria or markedly elevated peak urinary orotic acid following ingestion of allopurinol (Table I). Of the four remaining heterozygotes (confirmed by DNA testing), two (the proband's maternal aunts, II-5 and II-2) had mild symptoms and two (the mother, II-1 and maternal grandmother, I-1) were essentially asymptomatic. All four had glutamine levels at the upper range of normal. Of these four patients, the two mildly symptomatic aunts (II-5 and II-2) had an allopurinol test. Their allopurinol tests were very abnormal and practically indistinguishable from the symptomatic females. The four females (III-4, III-1, II-3, and II-4) and one male (III-5) with normal OTC alleles all had normal glutamine levels and those tested had normal orotic acids levels at baseline or after allopurinol ingestion (see Table I). Plasma levels of citrulline and arginine were slightly lower in the symptomatic individuals but they fell within the normal range and therefore were not diagnostic.

DISCUSSION

The identification of a large family with a severe "neonatal type" mutation causing OTC deficiency undiagnosed for several generations provided an opportunity to study the heterogeneity of clinical presentation in females heterozygous for the same mutation and of similar genetic background. Such a large pedigree is rare as most families with OTC deficiency are rather small. There was a delay in the diagnosis of this family for many years until a male with acute neonatal hyperammonemic coma brought the family to medical attention when the correct diagnosis was made. The symptomatic females in the family did have a significant family history of early male deaths indicating a possible X-linked disorder; however, the history was either not elicited in the repeated medical encounters or their symptoms were relatively non specific and hyperammonemia was not suspected. This report illustrates the importance of including OTC deficiency in the differential diagnosis of females who present with all or some of the following symptoms: cyclic vomiting, lethargy, central hypotonia, behavioral problems, mental retardation, and abnormal hair texture. In addition to the clinical history, a complete family history should be taken to inquire about male infant deaths. When the diagnosis of OTC deficiency is made, genetic counseling is essential to identify other family members at risk and to provide updated information with respect to risks, diagnostic tests, treatment, and prenatal diagnosis. In females who are confirmed carriers by molecular analysis, prenatal testing is an option.

It is interesting to note that more than half of the heterozygous females in this pedigree had clinical problems, most likely related to hyperammonemia (vomiting, behavioral and learning disabilities) and arginine deficiency (skin rashes and abnormal hair texture). A previous report on 61 female heterozygotes from 17 families (very likely to have many different mutations) [Batshaw et al., 1986], indicated that only about 18% of obligate heterozygotes are expected to show encephalopathic symptoms. This fraction is understandable based on random X-chromosome inactivation in the liver producing a mosaic pattern of OTC deficiency. Our finding of a higher proportion of symptomatic females in this family may have been a coincidence or it may be unique to the severe R141Q mutation which is known to completely abolish enzyme activity [Lee and Nussbaum, 1989]. Alternately, skewed "lyonization" may have occurred in this family due to a genetic predisposition.

It is well known that random determination of blood ammonia in OTC deficient females during periods of "well being" is very likely to yield normal results. In contrast, plasma glutamine levels are likely to be abnormally high in symptomatic females even during "medically uneventful" periods. It was striking that all clearly symptomatic females in this family had markedly elevated glutamine levels, while the asymptomatic females or those with equivocal symptoms had normal levels. Similarly, two of the symptomatic females had spontaneous orotic aciduria. All heterozygous females who were tested regardless of symptoms had an abnormal allopurinol test confirming that this test is likely to be reliable in severe mutations. However, in individuals with less severe mutations, the test is likely to produce false negative results (personal observation) and the heterozygous females for "milder" mutations are also less likely to be symptomatic.

Symptomatic OTC deficient females can be treated successfully with a protein restricted diet, citrulline, and, if necessary, ammonia scavenger drugs such as sodium benzoate, sodium phenylacetate, or sodium phenylbutyrate. It has been our experience that these

TABLE I. Clinical, Biochemical, and Molecular Data in a Family With OTC Deficiency*

Patient	Clinical symptoms	Plasma ammonia μmol/L (<35)	Plasma glutamine μmol/L (41–86)	Plasma citrulline μmol/L (0–80	Plasma arginine μmol/L (2–18)	Urine orotate mmol/mol Cr (<3.1)	Orotic acid allopurinol mmol/mol Cr (<13)	Genotype (+/+, +/Y)
II-5	Depression	15	81	3	8	0.7, 0.7	38.9	R141Q/+
III-6	Mental retardation behavioral disorder, abnormal hair, seizures, migraine	29	142	1	4	1.6, 3.5	45.9	R141Q/+
III-2	Learning disabilities, short stature, abnormal hair	21	125	2	4	0.3, 28.0	31.0	R141Q/+
III-3	Behavioral disorder	13	123	2	4	0.9, 0.7	60.1	R141Q/+
III-4	NA	10	59	3	6	0.6, 0.4	9.5	+/+
III-5	NA	18	63	3	7	0.2, 0.5	9.1	+/Y
II-1	None	12	72	4	7	0.7	ND	R141Q/+
III-1	NA	19	58	3	7	0.9	ND	+/+
II-2	Depression, protein restriction, abnormal hair	17	75	2	4	0.5	26.4	R141Q/+
II-3	NA	21	73	3	9	ND	ND	+/+
I-1	None	20	61	2	6	ND	ND	R141Q/+
II-4	NA	11	69	4	8	ND	ND	+/+

* Patients are identified by numbers corresponding to their designation on the pedigree illustration (Fig. 1).
NA, not applicable. ND, not done. Normal values or results are denoted in brackets. Some individuals had two "base line" orotic acid determinations.

patients on treatment rarely have severe hyperammonemia episodes and have a good prognosis providing the condition is diagnosed early, and hyperammonemia can be anticipated, treated, and reversed when the elevation is still mild and of little clinical consequence.

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